

Application No.: 10/804677
Docket No.: CL2346USNA

Page 2

Amendments / Listing of Claims

Claims 1 (Original). An isolated nucleic acid molecule encoding a carotenoid biosynthetic enzyme, selected from the group consisting of:

- (a) an isolated nucleic acid molecule encoding the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, and 12;
- (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; or
- (c) an isolated nucleic acid molecule that is complementary to (a) or (b).

Claim 2 (Original). The isolated nucleic acid molecule of Claim 1 selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, and 11.

Claim 3 (Original). An isolated nucleic acid fragment of Claim 1 isolated from *Pectobacterium*.

Claim 4 (Original). A polypeptide encoded by the isolated nucleic acid molecule of Claim 1.

Claim 6 (Original). The polypeptide of Claim 4 selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, and 12.

Claim 6 (Original). An isolated nucleic acid molecule as set forth in SEQ ID NO:18, comprising the *crtE*, *crtX*, *crtY*, *crtI*, *crtB* and *crtZ*, genes or an isolated nucleic acid molecule having at least 95% identity to SEQ ID NO:18, wherein the isolated nucleic acid molecule encodes all of the polypeptides *crtE*, *crtX*, *crtY*, *crtI*, *crtB* and *crtZ*.

Claim 7 (Original). An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a geranylgeranyl pyrophosphate synthase enzyme of at least 301 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO: 2;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

Claim 8 (Original). An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a zeaxanthin glucosyl transferase enzyme of at least 425 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO: 4;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

Claim 9 (Original). An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a lycopene cyclase enzyme of at least 388 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO: 6;

Application No.: 10/804677
Docket No.: CL2346USNA

Page 3

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

Claim 10 (Original). An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a phytoene desaturase enzyme of at least 493 amino acids that has at least 81% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO: 8;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

Claim 11 (Original). An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a phytoene synthase enzyme of at least 309 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO: 10;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

Claim 12 (Original). An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a β -carotene hydroxylase enzyme of at least 178 amino acids that has at least 77% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO: 12;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

Claim 13 (Original). A chimeric gene comprising the isolated nucleic acid molecule of any one of Claims 1 or 7-12 operably linked to suitable regulatory sequences.

Claim 14 (Original). A vector comprising the isolated nucleic acid molecule of Claim 6.

Claim 15 (Original). A transformed host cell comprising the chimeric gene of Claim 12.

Claim 16 (Original). A transformed host comprising the isolated nucleic acid molecule of claim 6.

Claim 17 (Currently Amended). The transformed host cell of Claim 16 or 16 wherein the host cell is selected from the group consisting of bacteria, yeast, filamentous fungi, algae, and green plants.

Claim 18 (Original). The transformed host cell of Claim 17 wherein the host cell is selected from the group consisting of *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Yarrowia*, *Rhodospiridium*, *Lipomyces*, *Salmonella*, *Bacillus*, *Acinetobacter*, *Zymomonas*, *Agrobacterium*, *Flavobacterium*, *Rhodobacter*, *Rhodococcus*, *Streptomyces*, *Brevibacterium*, *Corynebacteria*, *Mycobacterium*, *Escherichia*, *Pantoea*, *Pseudomonas*, *Methylomonas*, *Methylobacter*, *Methylococcus*, *Methylosinus*, *Methylochromium*, *Methylocystis*, *Alcaligenes*, *Synechocystis*, *Synechococcus*, *Anabaena*,

Application No.: 10/804677
Docket No.: CL2346USNA

Page 4

Thiobacillus, Methanobacterium, Klebsiella, Methylophilus, Methylobacillus, Methylobacterium, Hyphomicrobium, Xanthobacter, Paracoccus, Nocardia, Arthrobacter, Rhodopseudomonas, Torulopsis, Rhodotorula, and Phaffia.

Claim 19 (Original). A method for the production of carotenoid compounds comprising:

- (a) providing a transformed host cell comprising:
 - (i) suitable levels of farnesyl pyrophosphate; and
 - (ii) a set of nucleic acid molecules encoding the enzymes selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, and 12 under the control of suitable regulatory sequences;
- (b) contacting the host cell of step (a) under suitable growth conditions with an effective amount of a fermentable carbon substrate whereby a carotenoid compound is produced.

Claim 20 (Original). A method for the production of carotenoid compounds comprising:

- (a) providing a transformed host cell comprising:
 - (i) suitable levels of farnesyl pyrophosphate; and
 - (ii) a the isolated nucleic acid molecule of claim 6 under the control of suitable regulatory sequences;
- (b) contacting the host cell of step (a) under suitable growth conditions with an effective amount of a fermentable carbon substrate whereby a carotenoid compound is produced.

Claim 21 (Currently Amended). A method according to Claim 19 ~~or~~ 20 wherein the transformed host cell is selected from the group consisting of C1 metabolizing hosts, bacteria, yeast, filamentous fungi, algae, and green plants.

Claim 22 (Currently Amended). A method according to Claim 19 ~~or~~ 20 wherein the C1 metabolizing host is a methanotroph and the fermentable carbon substrate is selected from the group consisting of methane, methanol, formaldehyde, formic acid, methylated amines, methylated thiols, and carbon dioxide.

Claim 23 (Original). A method according to Claim 22 wherein the C1 metabolizing host:

- (a) grows on a C1 carbon substrate selected from the group consisting of methane and methanol; and
- (b) comprises a functional Embden-Meyerhof carbon pathway, said pathway comprising a gene encoding a pyrophosphate-dependent phosphofructokinase enzyme.

Application No.: 10/804677
Docket No.: CL2346USNA

final

Page 5

Claim 24 (Original). A method according to Claim 23 wherein the C1 metabolizing host cell is a high growth methanotrophic bacterial strain, known as *Methylomonas* 16a and having the ATCC designation PTA 2402.

Claim 25 (Currently Amended). A method according to Claim 19-~~or~~ 20 wherein the transformed host cell is selected from the group consisting of *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Yarrowia*, *Rhodospiridium*, *Lipomyces*, *Salmonella*, *Bacillus*, *Acinetobacter*, *Zymomonas*, *Agrobacterium*, *Flavobacterium*, *Rhodobacter*, *Rhodococcus*, *Streptomyces*, *Brevibacterium*, *Corynebacteria*, *Mycobacterium*, *Escherichia*, *Pantoea*, *Pseudomonas*, *Methylomonas*, *Methylobacter*, *Methylococcus*, *Methylosinus*, *Methylomicrobium*, *Methylocystis*, *Alcaligenes*, *Synechocystis*, *Synechococcus*, *Anabaena*, *Thiobacillus*, *Methanobacterium*, *Klebsiella*, *Methylophilus*, *Methylobacillus*, *Methylobacterium*, *Hyphomicrobium*, *Xanthobacter*, *Paracoccus*, *Nocardia*, *Arthrobacter*, *Rhodopseudomonas*, *Torulopsis*, *Rhodotorula*, and *Phaffia*.

Claim 26 (Currently Amended). A method according to Claim 19-~~or~~ 20, wherein the carotenoid compound produced is selected from the group consisting of: antheraxanthin, adonirubin, adonixanthin, astaxanthin, canthaxanthin, capsorubrin, β -cryptoxanthin, α -carotene, β -carotene, epsilon-carotene, echinenone, 3-hydroxyechinenone, 3'-hydroxyechinenone, γ -carotene, 4-keto- γ -carotene, ζ -carotene, α -cryptoxanthin, deoxyflexixanthin, diatoxanthin, 7,8-didehydroastaxanthin, fucoxanthin, fucoxanthinol, isorenieratene, lactucaxanthin, lutein, lycopene, myxobactone, neoxanthin, neurosporene, hydroxynurosporene, peridinin, phytoene, rhodopin, rhodopin glucoside, 4-keto-rubixanthin, siphonaxanthin, spheroidene, spheroidenone, spirilloxanthin, 4-keto-torulene, 3-hydroxy-4-keto-torulene, uriolide, uriolide acetate, violaxanthin, zeaxanthin- β -diglucoside, and zeaxanthin.

Claim 27 (Original). A method of regulating carotenoid biosynthesis in an organism comprising over-expressing at least one carotenoid gene selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, and 18 in an organism such that the carotenoid biosynthesis is altered in the organism.

Claim 28 (Original). A method according to Claim 27 wherein said carotenoid gene is over-expressed on a multicopy plasmid.

Claim 29 (Original). A method according to Claim 27 wherein said carotenoid gene is operably linked to an inducible or regulated promoter.

Claim 30 (Original). A method according to Claim 27 wherein said carotenoid gene is expressed in antisense orientation.

Claim 31 (Original). A method according to Claim 27 wherein said carotenoid gene is disrupted by insertion of foreign DNA into the coding region.

Claim 32 (Original). A *Pectobacterium* sp. comprising the 16s rDNA sequence as set forth in SEQ ID NO:16.